Detection limit of Mycobacterium chimaera in water samples for monitoring medical device safety: insights from a pilot experimental series.

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Abstract: OBJECTIVE: A growing number of Mycobacterium chimaera infections after cardiosurgery have been reported by several countries. These often fatal infections were traced back to contaminated heater-cooler devices (HCDs), which use water as heat transfer medium. Aerosolization of water contaminated with M. chimaera from HCDs enables airborne transmission to patients undergoing open chest surgery. Infection control teams test HCD water samples for mycobacterial growth to guide preventive measures. The detection limit of M. chimaera in water samples, however, has up to now not been investigated. METHODS: A M. chimaera strain representative of the international cardiosurgery associated M. chimaera outbreak was used to generate a logarithmic dilution series. Two different water volumes, 50ml and 1000ml, were inoculated and after identical processing (centrifugation, decantation, and decontamination) seeded on Mycobacteria growth indicator tube (MGIT) and Middlebrook 7H11 solid media. RESULTS: MGIT consistently showed a lower detection limit as 7H11 solid media, corresponding to a detection limit of a concentration of 1.44x104 CFU/ml for 50ml and 2.4 CFU/ml for 1000ml water samples. Solid media failed to detect M. chimaera in 50ml water samples. CONCLUSION: Depending on water volume and culture method, major differences exist in the detection limit of M. chimaera. In terms of sensitivity, 1000ml water samples in MGIT media performed best. Our results have important implications for infection prevention and control strategies in mitigation of the M. chimaera outbreak and healthcare water safety in general.

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Detection limit of *Mycobacterium chimaera* in water samples for monitoring medical device safety: insights from a pilot experimental series

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SUMMARY

Background: A growing number of *Mycobacterium chimaera* infections after cardiosurgery have been reported by several countries. These potentially fatal infections were traced back to contaminated heater–cooler devices (HCDs), which use water as a heat transfer medium. Aerosolization of water contaminated with *M. chimaera* from HCDs enables airborne transmission to patients undergoing open chest surgery. Infection control teams test HCD water samples for mycobacterial growth to guide preventive measures. The detection limit of *M. chimaera* in water samples, however, has not previously been investigated.

Aim: To determine the detection limit of *M. chimaera* in water samples using laboratory-based serial dilution tests.

Methods: An *M. chimaera* strain representative of the international cardiosurgery-associated *M. chimaera* outbreak was used to generate a logarithmic dilution series. Two different water volumes, 50 and 1000 mL, were inoculated, and, after identical processing (centrifugation, decantation, and decontamination), seeded on mycobacteria growth indicator tube (MGIT) and Middlebrook 7H11 solid media.

Findings: MGIT consistently showed a lower detection limit than 7H11 solid media, corresponding to a detection limit of $\geq 1.44 \times 10^4$ cfu/mL for 50 mL and ≥ 2.4 cfu/mL for 1000 mL water samples. Solid media failed to detect *M. chimaera* in 50 mL water samples.

Conclusion: Depending on water volume and culture method, major differences exist in the detection limit of *M. chimaera*. In terms of sensitivity, 1000 mL water samples in MGIT media performed best. Our results have important implications for infection prevention and control strategies in mitigation of the *M. chimaera* outbreak and healthcare water safety in general.

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Introduction

Mycobacterium chimaera, a member of the *Mycobacterium avium* complex first described in 2004, recently stirred the awareness of hospital epidemiologists and infectious disease specialists due to its association with infections following cardiosurgery [1]. Since the description of the first two cases in 2013, an increasing number of *M. chimaera* infections associated with cardiosurgery interventions has been reported from numerous countries [2–8]. *M. chimaera*, other non-tuberculous mycobacteria (NTM) as well as other types of bacteria have been isolated from the water system of heater–cooler devices (HCDs) [9–12]. Also, air samples collected next to operating HCDs that were contaminated with *M. chimaera* grew *M. chimaera* [13,14]. Aerosols containing *M. chimaera* are being generated in the water reservoir of HCDs and subsequently dispersed by a fan used for heat exchange [13]. Airborne transmission from contaminated HCDs to implants and the surgical site during cardiac surgery is considered the most likely route of infection [15]. Endovascular or disseminated infections caused by *M. chimaera* may be characterized by delayed diagnosis and poor prognosis [16]. Despite aggressive antibiotic therapy and revision surgery, curability remains uncertain, as relapses may occur even after prolonged antimicrobial therapy [16,17] (B. Hasse, Zurich, Switzerland, personal communication).

Due to their clinical relevance, surveillance cultures from HCD water samples are often employed to assess efficacy of decontamination procedures [10,13,18]. However, no data have been published on the detection threshold of *M. chimaera* in water samples. We sought to close this gap with laboratory-based serial dilution tests.

Methods

Stock solution, dilution series 1–8

The isolate chosen for this investigation was *M. chimaera* ZUERICH-1 (DSM 101591). The genome of the strain has been sequenced completely (NCBI GenBank accession NZ_CP015272) and is characterized by a median pairwise distance of only 12 single nucleotide polymorphisms in comparison to isolates gathered from LivaNova (London, UK; formerly Sorin or Stöckert) heater–cooler units; thus, it is highly related to the strains recovered from HCDs and infected patients [12]. After five days of incubation in a mycobacteria growth indicator tube (MGIT 960; Becton Dickinson, Sparks, MD, USA), a suspension of 0.5 McFarland, corresponding to 10^7 cfu/mL, was generated in 5 mL sterile water [19]. Five-day growth cultures were chosen to gain mycobacteria while showing logarithmic growth behaviour. Tube 1 contained the undiluted suspension. Tubes 2–8 were prefilled with 36 mL sterile water each, to create a logarithmic dilution series, i.e. dilutions 2–8. Dilutions 2–8 resulted from adding 4 mL of the solution from one tube into the next consecutive tube, i.e. dilution 2 was generated by adding 4 mL of dilution 1 to 36 mL of sterile water in tube 2, the dilution 3 by adding 4 mL of dilution 2 to 36 mL sterile water in tube 3, etc (Figure 1). To reduce clumping of mycobacteria, the initial solution for each dilution was dispersed by drawing up and expelling 10 times through a 26-gauge needle attached to a 1 mL syringe [20].

To verify the resulting concentrations, 0.1 mL of dilutions 4–8 were seeded on 7H11 solid media (BD Difco Mycobacteria

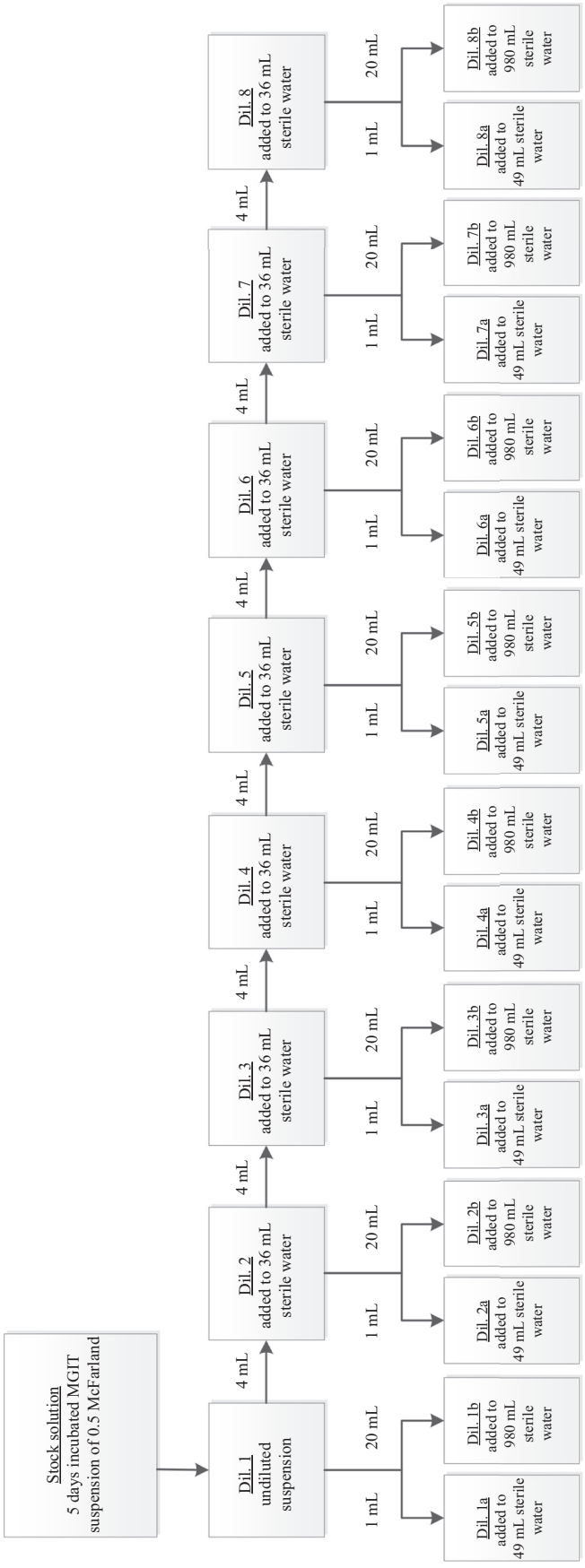

Figure 1. Dilution series. MGIT, mycobacterial growth indicator tube; Dil., dilution.

7H11 Agar; Becton Dickinson) with 10% OADC (oleic albumin dextrose catalase; Becton Dickinson) in triplicates and incubated at 37 ± 1.5°C and 5–10% CO_2 for seven weeks or until positive. Solid media agar plates were sealed with Parafilm M (Bemis, Neenah, WI, USA) to prevent drying. Concentrations of dilutions 1–3 were calculated based on the results of dilution 4. Due to the logarithmic dilution, multiplication by 10 was necessary to calculate each precedent number of colony-forming units (cfu) and resulting concentrations. Cultures were assessed weekly. In case of growth, colonies were counted and identified as mycobacteria by acid-fast staining. Upon confirmation, colonies were suspended in 3 mL 0.9% sodium chloride. According to the instructions of the manufacturer, 0.5 mL of this suspension underwent molecular identification using InstaGene Matrix (Bio-Rad, Hercules, CA, USA). A modified LightCycler 16S rDNA assay was performed to identify non-tuberculous mycobacteria [21]. If the *Mycobacterium* sp. probe was positive, the LightCycler polymerase chain reaction amplicon was purified and subjected to sequencing. SmartGene IDNS 16S rDNA database (SmartGene Zug, Switzerland) was used for comparisons of 16S rDNA homology, enabling species identification.

Water samples

Water samples of 50 mL; dilution series 1a–8a

For each 50 mL water sample, 1 mL of dilutions 1–8 was added to each 49 mL sterile water, resulting in the dilutions 1a–8a (Figure 1). Samples were centrifuged at 3300 *g* for 15 min; after centrifugation samples were decanted and resuspended with sterile water until 5 mL left-over including pellet remained in the primary tube. The decontamination method for inactivation of non-mycobacterial species in water samples has been evaluated before setting up this study (Swiss National Center for Mycobacteria, University Zurich, data not shown). Samples have been decontaminated as follows: 5 mL decontamination solution (BBL MycoPrep Kit, BD, Franklin Lakes, NJ, USA) was added to the primary tube. After 15 min, 30 mL phosphate buffer (BBL MycoPrep Kit) was added. After decontamination a volume of 500 μL was used for MGIT and 100 μL were seeded on 7H11 solid media. Dilutions 6a–8a were seeded undiluted; dilution 5a both undiluted and diluted to 1:10 (using phosphate buffer); dilution 4a diluted both to 1:10 and 1:100; dilution 3a diluted both to 1:100 and 1:1000; dilution 2a both to 1:1000 and 1:10,000; and dilution 1a both to 1:10,000 and 1:100,000. Assessment of solid media for growth and identification was performed as described above for the dilution series 1–8.

Water samples of 1000 mL; dilution series 1b–8b

For each 1000 mL water sample, 20 mL of dilutions 1–8 were added to each 980 mL sterile water, resulting in dilutions 1b–8b (Figure 1). Centrifugation, decantation, resuspension and decontamination procedures were performed as described above for the 50 mL water samples. After decontamination, 500 μL was used to inoculate MGIT tubes and 100 μL for 7H11 solid media. Dilutions 7b–8b were seeded undiluted; dilution 6b both undiluted and diluted to 1:10 (using phosphate buffer); dilution 5b diluted both to 1:10 and 1:100; dilution 4b diluted both to 1:100 and 1:1000; dilution 3b diluted both to 1:1000 and 1:10,000; dilution 2b diluted both to 1:10,000 and 1:100,000; and dilution 1b diluted both to 1:100,000 and 1:1,000,000. Assessment of solid media for growth and identification was performed as described above for the dilution series 1–8.

Calculation of the detection limit

For determination of the detection limit the highest dilution resulting in growth of *M. chimaera* was considered for both volumes and methods, respectively. The concentration responding to the detection limit was calculated by dividing the initial inoculum by the total volume.

Results

Bacterial concentrations in stock solution and dilution series

Dilutions 4–8 showed a median count of 720, 131, 36, 12, and 21 cfu/100 μL, equalling concentrations of 7200, 1310, 360, 120, and 210 cfu/mL, respectively (Table I). For dilutions 1, 2, and 3, concentrations of 7.2×10^6, 7.2×10^5, and 7.2×10^4 cfu/mL, respectively, were extrapolated based on the concentration of dilution 4.

Detection of *M. chimaera* in the two investigated water sample volumes

Water samples of 50 mL

With water samples of 50 mL, MGIT detected *M. chimaera* only in the highest two concentrations tested, i.e. dilution 1a and 2a, corresponding to a detection limit of a bacterial

Table I
Quantification of stock solution and logarithmic dilution series

Quantification	Dilution							
	1	2	3	4	5	6	7	8
cfu count 1	nd	nd	nd	688	115	35	12	16
cfu count 2	nd	nd	nd	720	131	36	12	21
cfu count 3	nd	nd	nd	792	140	48	25	26
Median cfu count	720,000[a]	72,000[a]	7200[a]	720	131	36	12	21
Concentration (cfu/mL)	7,200,000	720,000	720,00	7200	1310	360	120	210

cfu, colony-forming units; nd, not done.
A negative control showed no growth in MGIT liquid media and on 7H11 solid media.
cfu count reported for seeding of 100 μL of the corresponding dilutions on 7H11 solid media.
[a] Extrapolated from dilution 4.

concentration of $\geq 1.44 \times 10^4$ cfu/mL (Table II). In dilution 1a (inoculum of 1.44×10^5 cfu/mL), growth of *M. chimaera* was reported five days earlier than in dilution 2a (inoculum of 1.44×10^4 cfu/mL). *M. chimaera* did not grow at all on 7H11 solid media in any of the test dilutions.

Water samples of 1000 mL

When water samples of 1000 mL were used, MGIT was able to identify *M. chimaera* in all tested dilutions, equalling a detection limit of a bacterial concentration of ≥ 2.4 cfu/mL (Table III). When comparing the proportion of positive culture results in MGIT, there was a significant difference between 50 and 1000 mL water samples (Fisher's exact test, $P = 0.007$). The 7H11 solid media detected *M. chimaera* in dilution 1b (inoculum of 1.44×10^5 cfu/mL; two colonies) and dilution 5b (inoculum of 26.2 cfu/mL, one colony), but not in dilutions 2b–4b.

Discussion

By comparing 7H11 and MGIT media for mycobacterial culture and by comparing two different water sample volumes of 50 and 1000 mL, we gathered relevant insights on the detection limit of *M. chimaera* in water. The sensitivity of MGIT for detecting *M. chimaera* in centrifuged 1000 mL water samples was excellent by contrast with centrifuged 50 mL water samples, with a detection limit of >10,000 cfu/mL.

The use of 7H11 solid media proved unsuitable for *M. chimaera* detection for either of the two sampling volumes. Nevertheless, due to its broader growth conditions, 7H11 solid media offer the advantage of detecting other bacterial species, which have been also reported in HCDs [22]. In the 1000 mL dilution series, growth of *M. chimaera* on 7H11 solid media was inconsistent. This observation might be explained by a stochastic phenomenon. Clump formation or cording of *M. chimaera* in the dilutions could have hindered adequate inoculation of 7H11 solid media. However, we cannot confirm this hypothesis, as we did not perform microscopy in these samples. For the present study, we decided to use 7H11 media sealed with Parafilm M and not Lowenstein–Jensen tubes as solid media. 7H11 media allow the recovery of mycobacteria more easily and previous studies indicated non-inferior recovery rates for NTMs, if desiccation of the media was prevented by Parafilm M [21]. In terms of sensitivity, 1000 mL water sampling combined with MGIT represents the method of choice to detect *M. chimaera* from environmental water sources such as water-bearing medical devices including HCDs, extracorporeal circuit membrane oxygenators (ECMOs) as well as water fountains, and tap water. One barrier in implementation may be the need for large-volume centrifuges. However, if water samples are tested to assist in risk management decision, the significantly increased sensitivity of 1000 mL samples should promote motivation for acquisition of corresponding equipment. If the water volume in the source to be tested is <1000 mL, 50 mL water samples and MGIT media may be employed, while recognizing the considerably lower sensitivity of this approach. If sample volumes between 50 and 1000 mL were used, feasibility would be even more complex, as pooling and multiple centrifuge runs would be required. According to a recent report, it took six months until *M. chimaera* could be isolated from factory-new HCDs that were most likely already contaminated during their production [10].

Table II
Culture results for dilution series of 50 mL water samples

Inoculum/culture	Dilution							
	1a	2a	3a	4a	5a	6a	7a	8a
Inoculum (cfu)	7,200,000	720,000	72,000	7200	1310	360	120	210
MGIT (time until positive, days)	Pos (12)	Pos (17)	Neg	Neg	Neg	Neg	Neg	Neg
7H11 (cfu counted)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
7H11 (cfu/mL)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

cfu, colony-forming unit; MGIT, mycobacterial growth indicator tube.
All dilutions were plated once.
A negative control showed no growth in MGIT liquid media and on 7H11 solid media.

Table III
Culture results for dilution series of 1000 mL water samples

	Dilution							
	1b	2b	3b	4b	5b	6b	7b	8b
Inoculum (cfu)	1.44×10^8	1.44×10^7	1,440,000	144,000	26,200	7200	2400	4200
MGIT (time until positive, days)	Pos (5)	Pos (7)	Pos (9)	Pos (15)	Pos (11)	Pos (18)	Pos (17)	Pos (25)
7H11 (cfu counted)	2[a]	Neg	Neg	Neg	1[b]	Neg	Neg	Neg
7H11 (cfu/mL)[c]	2×10^6	Neg	Neg	Neg	1×10^7	Neg	Neg	Neg

cfu, colony-forming unit; MGIT, mycobacterial growth indicator tube.
The dilutions were plated once.
A negative control showed no growth in MGIT liquid media and on 7H11 solid media.
[a] Seeded 1:100,000 diluted.
[b] Seeded 1:10 diluted.
[c] Calculated based on cfu count on 7H11.

Hypothetically, this observation may be due to a certain timespan having been needed for mycobacterial growth until the detection limit of 50 mL samples was reached.

A single study tested *M. chimaera* contamination of HCDs using swabs from surfaces and tubings of ECMOs [23]. Sampling of inner surfaces of tubes or tanks could be taken into consideration as an alternative method to assess the contamination status of HCDs. However, gaining representative samples requires partial disassembly of HCDs, thus complicating regular testing. There are currently no data on the relative sensitivity comparing the two methods.

Routine surveillance cultures of HCD water for mycobacteria were discussed controversially with the main argument being the undefined negative predictive value due to the unknown limit of detection [24,25]. With respect to feasibility, Total viable counts (TVCs) were frequently used to assess contamination of HCDs in general. In a recent study with repeated determination of TVCs and selected samples for mycobacterial cultures, presence of *M. chimaera* was only described if TVCs were high [26]. With respect to the coexistence of *M. chimaera* and other pathogens in biofilms, a positive correlation between TVCs and presence of *M. chimaera* might be hypothesized, but up to now there are insufficient data to support this hypothesis. Notably, in this study, water samples of 100 mL were used, which are likely inferior to 1000 mL samples in terms of sensitivity. Awareness of the detection limit of *M. chimaera* in HCD water samples is relevant for the mitigation of the current outbreak associated with cardiac surgery [14]. An early and reliable detection of NTM contamination of water-containing medical devices is pivotal to develop a safe technical solution and assists in effective management of these devices in clinical use. Our results should also be considered in guidelines for HCD management.

In environmental microbiology, limit-of-detection assays have been established mainly for *Legionella* spp. [27]. For mycobacterial detection in environmental samples, only relatively small studies showing widely varying results have been reported, e.g. a few studies demonstrated the occurrence of opportunistic NTM species in environmental water samples [28–30]. A study by Radomski *et al.* investigated the effect of different decontamination methods and culture media supplementation with antibiotics in the detection of NTM from environmental samples [31].

A major strength of our study is the use of the purposefully selected *M. chimaera* strain. Due to its clinical relevance, an *M. chimaera* strain from a patient infected during cardiosurgery in the context of the current global outbreak was chosen. Strikingly, in a recent study using whole genome sequencing, this strain clustered with all isolates except one gathered from individuals suffering from HCD-associated *M. chimaera* infection, the vast majority of *M. chimaera* isolates from LivaNova HCDs in use, and an isolate derived from the LivaNova manufacturing site [12].

This study has limitations. Our experiments focused on a single – but in the context representative – *M. chimaera* strain. With respect to the limited number of experiments, our results are of pilot character. The concentration of *M. chimaera* in HCD water systems that is associated with an infectious risk for patients is unknown. This risk, however, certainly depends on multiple additional factors beyond bacterial concentration in HCD water, including HCD design features, HCD positioning and orientation, operating room ventilation profile, and *M. chimaera* strain properties. Notably, besides *M. chimaera*, other NTM such as *M. abscessus* have been associated with HCD-derived infections [11]. Similar experiments with other NTM are lacking, but in a study on HCD surveillance we cultured several NTM species in 50 mL water samples using the methods described [10]. Until sounder evidence becomes available, any contamination of HCD water with *M. chimaera* and other NTM should trigger preventive measures to guarantee patient safety [11,24]. Decontamination procedures are complicated by biofilm formation within HCDs, enabling persistence of *M. chimaera* [17,18]. Furthermore, air culture sensitivity for NTM remains unknown despite its central importance for HCD safety. Studying microbial detection limits in air volumes is notoriously challenging [32,33] and was beyond the scope of the current protocol.

In conclusion, our study showed major differences in the detection limit for *M. chimaera* for the assessed laboratory protocols. Highest sensitivity was achieved by 1000 mL water samples in MGIT media, and solid media failed to detect *M. chimaera* in 50 mL water samples. These findings have important implications for infection prevention and control strategies regarding the infectious risk associated with NTM and stagnant water in healthcare settings. Current international recommendations on the management of HCD may have to be adapted accordingly.

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Conflict of interest statement
None declared.

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